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BEFORE THE BOARD OF APPEALS AND INTERFERENCES
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Arnold et al.

Serial No. 09/586,156

Filed: June 2, 2000

For: *Duplex Probes for Hybridization Reactions*

Group Art Unit: 1655

Examiner: Lu, F.

Attorney Docket No. IN-0016-1

CERTIFICATE OF MAILING
I hereby certify that this corr is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to the Comm of Patents, Washington, D.C. 20231 on July 11, 2001

Signed _____
Richard Osman

BRIEF ON APPEAL

The Honorable Board of Appeals and Inteferences
United States Patent and Trademark Office
Washington, D.C. 20231

Dear Honorable Board:

This is an appeal from the Feb 15, 2001 Final Rejection of claims 1-24.

REAL PARTY IN INTEREST

The real party in interest is Incyte Genomics, Inc., the assignee of this patent application.

RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related appeals or interferences.

STATUS OF THE CLAIMS

Claims 1-24 are pending; claims 4-7 were deemed allowable if rewritten in independent form; hence, claims 1-3 and 8-24 are subject to this appeal.

STATUS OF THE AMENDMENTS

Our amendment to claim 15 submitted 6/22/01 was entered pursuant to the Advisory Action transmitted 7/10/01. Accordingly, all Amendments are believed to be properly before the Board.

SUMMARY OF THE INVENTION

The invention is a solid phase hybridization assay. In one embodiment, the invention is directed to a method for immobilizing a polynucleotide probe by: combining the probe with a polynucleotide target stably associated with a surface of a solid support, wherein one of the probe and target is double-stranded comprising complementary strands, and the other is single-stranded having complementarity with one of the complementary strands, under conditions wherein the probe and target hybridize and the probe is thereby immobilized. Specification, claim 1 as filed, p.19, lines 2-7.

In particular embodiments subject to dependent claims, the method further comprises the step of detecting specific hybridization of the probe and the target (claim 2), or releasing the immobilized probe (claim 3). In other particular embodiments subject to dependent claims, the double-stranded probe or target comprises noncovalently (claim 8) or covalently linked complementary strands (claim 9); the probe comprises a detectable label (claim 10); the target and solid support are of a microarray (claim 11); the recited conditions include an amount of a metal ion sufficient to enhance hybridization of the target and probe (claim 12), particularly wherein the metal ion is selected from the group consisting of Zn++, Ni++, Ca++, Fe++, Fe+++, Mg++, K+, Co++ and Co+++ (claim 13); or the solid support comprises a polycationic surface (claim 14). Specification, Examples 3-10, p.13, line 7 - p.18, line 10.

In another embodiment, the invention is directed to a method for immobilizing and detecting a polynucleotide probe by: contacting a polynucleotide target which is stably associated with a surface of a solid support, with a double-stranded polynucleotide probe under conditions wherein the double-stranded probe hybridizes with the target; and detecting specific hybridization of the double-stranded probe to the target. Specification, claim 15 as filed, p.20, lines 20-24.

In particular embodiments subject to dependent claims, the probe comprises noncovalently (claim 16) or covalently (claim 17) linked complementary strands; covalently linked complementary strands and is of hairpin structure (claim 18); covalently linked complementary strands, linked

through a first coupling moiety on one strand and a second coupling moiety on the second strand (claim 19), particularly wherein (a) the first coupling moiety is an alkylamine and the second coupling moiety is selected from an active ester, amide, imine, aldehyde, bromoacetamide and thiocyanate; or (b) the first coupling moiety is a thiol and the second coupling moiety is selected from a disulfide, maleimides and bromoacetamides (claim 20); or a detectable label (claim 21). In other particular embodiments subject to dependent claims, the target is single-stranded (claim 22); the target and solid support are of a microarray (claim 23); or the solid support comprises a polycationic surface (claim 24). Specification, Examples 3-10, p.13, line 7 - p.18, line 10.

ISSUES

I. WHETHER CLAIMS 1, 2, 8, 11, 15, 16, 22 and 23 ARE PATENTABLE UNDER 35USC102(b).

II. WHETHER CLAIMS 1, 2, 8-13 and 15-23 ARE PATENTABLE UNDER 35USC103(a).

III. WHETHER CLAIM 3 IS PATENTABLE UNDER 35USC103(a).

IV. WHETHER CLAIMS 14 and 24 ARE PATENTABLE UNDER 35USC103(a).

GROUPING OF THE CLAIMS

For Issue I, claims 1, 2, 8, 11, 15, 16, 22 and 23 shall stand together as a group.

For Issue II, 1, 2, 8-13 shall be considered as group; and claims 15-23 shall be considered a separate group.

For Issue III, claim 3 shall be considered separately.

For Issue IV, claim 14 shall be considered separately; and claim 24 shall be considered separately.

ARGUMENT

I. CLAIMS 1, 2, 8, 11, 15, 16, 22 and 23 ARE PATENTABLE UNDER 35USC102(b).

The structural requirements of our claims are neither met nor suggested by the cited Bates et al. (Nucleic Acids Res. 23, 3627-3632, 1995), which describes the entirely unrelated Hoogsteen triplex formation (p.3628, col.1, line 16). The three triplex-forming systems of Bates are shown in her Table 1: the cited reactions are (1)Bt-T30/A30/T30, (2)Bt-AY/AU/Pso-20 and (3)Bt-HD1/HD2/HD3. Figure 2 describes each of these molecules: in each case, you have the third single

stranded molecule binding the backside of the purines of the immobilized double stranded molecule. Note that the third strands are always pyrimidine polymers (T30, Pso-20 or HD3; see Fig.2). There is no complementarity[1], as expressly required by our claims. Note that even with Bates' polyT/polyA homopolymers, the orientation of the polyT probe binding is parallel (not complementary) with the polyA of the duplex (see Fig.2) and the polyA probe binding of the A30-A30-T30 triplex (p.3630) is antiparallel with the polyA - not with the polyT.[2] Bates' solid phase Hoogsteen binding assay provides a useful system for studying the kinetics of this phenomenon, however it is inapplicable to other than pyrimidine probes and purine rich targets, and it is not a hybridization assay as claimed. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

II. CLAIMS 1, 2, 8-13 and 15-23 ARE PATENTABLE UNDER 35USC103(a).

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996) and Pease et al. (Proc. Natl. Acad. sci. USA 91, 5022-5026, 1994). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the

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[1] The Action appears to rely on an untenable construction of our claims, construing the requirement of "having complementarity" to mean merely having "a potential to be complementary". We believe the complementarity limitation of our claim is unconditional. We do not understand the Action's motivation for restating our claims, nor do we know the source of the proposed alternative language, nor do we understand what is intended or meant by "potential to be complementary" - as a molecule either has complementarity or it does not. Perhaps the Action is inadvertently equating complementary with hybridized polynucleotides? The rejection seems more directed to a claim reciting conditional functional language like "capable of hybridizing with".

[2] Though not germane to the present rejection, to the extent the Board elects to carefully dissect the Bates reference, we note that Bates twice erroneously refers to a "HD1-HD2·HD3" system (at p.3630, col.2), when in fact, by Bates' nomenclature (p.3628, first full para.), the referenced system is properly called "HD3-HD2·HD1", i.e. HD3 is Hoogsteen bound to the HD2·HD1 duplex (p.3229, Fig.3C; p.3631, Fig.6; and p.3631, first full para.).

probe and target keeps the arms (and the attached fluorescent and quenching moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the probe).

The cited Pease reference is no more than an introduction to microarrays generated by the now well-known method of photolithography. Pease also exclusively teaches conventional single strand - single strand hybridization (e.g. Pease, p.5024, paragraph bridging col.1 and 2). Combining the two references would provide no more than the use of Tyagi's doubly labeled probes in a conventional single strand - single strand hybridization reaction. Nowhere described or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

Claims 15-23 are further removed from the cited art as these claims expressly require that the probe which hybridizes to the immobilized target be double-stranded. The cited art suggests nothing but single-stranded molecules hybridizing to single-stranded target. Though Tyagi's probes can exist in a partially-double stranded form, they are not double-stranded when hybridized to target - in fact, the target hybridizing portion of Tyagi's probes do not have a complementary sequence within the probe.

III. CLAIM 3 IS PATENTABLE UNDER 35USC103(a).

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996) and Anderson et al (1985 Nucleic Acid Hybridization: a practical approach p. 86-109). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms

anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the probe and target keeps the arms (and the attached fluorescent and quenching moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the probe).

The Anderson reference is cited for no more than the reuse of filters and probes after hybridization. Combining the two references would provide no more than the reuse of Tyagi's doubly labeled probes in a conventional single strand - single strand hybridization reaction. Nowhere described or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

IV. CLAIMS 14 and 24 ARE PATENTABLE UNDER 35USC103(a).

This art rejection cites Tyagi et al. (Nature Biotech. 14, 303-308, 1996), Pease et al. (Proc. Natl. Acad. sci. USA 91, 5022-5026, 1994) and Brown et al. (US Patent No. 5,807522). Tyagi describes self quenching probes that fluoresce only upon hybridization to a target. The probes have a target complementary sequence flanked by complementary (to each other) arm sequences, one arm labeled with a fluorescent moiety and the other with a non-fluorescent quencher. In the absence of target, the arms anneal, forming a stem-loop structure and fluorescence is quenched by the resultant proximity of the fluorescent moiety and quenching moiety. However, in the presence of target, the hybridization between the probe and target keeps the arms (and the attached fluorescent and quenching moeties) separated, and thereby prevents quenching.

Tyagi describes a clever probe labeling system, but neither discloses nor suggests anything about the claimed duplex hybridization method. Tyagi relies on conventional single strand - single

strand hybridization. Note that Tyagi's complementary arm sequences are necessarily unrelated to the target sequence (e.g. p.303, col.1, line 30). Hence, Tyagi can not meet or suggest (and necessarily teaches directly away from) our method's requirement that the single stranded component (here, the target) have complementarity with one of the strands of the double-stranded component (here, the probe).

The cited Pease reference is no more than an introduction to microarrays generated by the now well-known method of photolithography. Pease also exclusively teaches conventional single strand - single strand hybridization (e.g. Pease, p.5024, paragraph bridging col.1 and 2). Brown is cited for the use of poly-L-lysine to coat microarray substrates. Combining the three references would provide no more than the use of Tyagi's doubly labeled probes in a conventional single strand - single strand hybridization reaction on a substrate coated with poly-L-lysine. Nowhere described or suggested is the claimed solid phase hybridization assay involving triplex formation by hybridization between complementary targets and probes. Absent such a prior art teaching or suggestion, the claims are in compliance with 35USC102 and 103.

Claim 24 is further removed from the cited art as this claim expressly requires that the probe which hybridizes to the immobilized target be double-stranded. The cited art suggests nothing but single-stranded molecules hybridizing to single-stranded target. Though Tyagi's probes can exist in a partially-double stranded form, they are not double-stranded when hybridized to target - in fact, the target hybridizing portion of Tyagi's probes do not have a complementary sequence within the probe.

CONCLUSION

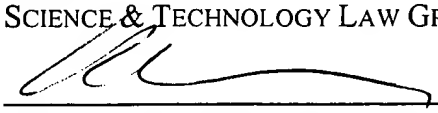
The purportedly responsive remarks contained in the Advisory Actions mailed 7/5/01 and transmitted 7/10/01 are either not comprehensible to us (p.2, lines 13-19), inaccurate (p.3, lines 1-2; Bates can not disclose a A30-T30·A30 because she does not even use a biotinylated A30; see Fig.2), or not relevant to our claims (p.3, lines 10-14 and 18-20).

Our claims are duly limited to a solid phase hybridization assay: an immobilized target hybridizes with a probe, wherein one of the two is double-stranded and the other is single-stranded. The single-stranded molecule is complementary to one of the strands of the double-stranded molecule, i.e. it has the complementary sequence and opposite orientation as one strand and the

same sequence and orientation as the other strand of the double-stranded molecule. As explained above, this structural requirement is neither met nor suggested by the cited art. In fact, the invention is premised on and limited to a hitherto unexploited binding phenomenon, distinct from Hoogsteen binding of the cited Bates reference, and the conventional single stranded - single stranded hybridization reactions of the Tyagi, Pease, Brown and Anderson references. Applicants respectfully request reversal of the pending Final Action by the Board of Appeals.

Applicants hereby petition for any necessary extension of time pursuant to 37 CFR 1.136(a). The Commissioner is hereby authorized to charge any necessary fees associated with this communication to our Deposit Account No. 19-0750 (order no. IN-006-1US).

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP



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CLAIMS ON APPEAL

1. A method for immobilizing a polynucleotide probe comprising the steps of: combining the probe with a polynucleotide target stably associated with a surface of a solid support, wherein one of the probe and target is double-stranded comprising complementary strands, and the other is single-stranded having complementarity with one of the complementary strands, under conditions wherein the probe and target hybridize and the probe is thereby immobilized.

2. A method according to claim 1, further comprising the step of detecting specific hybridization of the probe and the target.

3. A method according to claim 1, further comprising the step of releasing the immobilized probe.

8. A method according to claim 1, wherein the double-stranded probe or target comprises noncovalently linked complementary strands.

9. A method according to claim 1, wherein the double-stranded probe or target comprises covalently linked complementary strands.

10. A method according to claim 1, wherein the probe comprises a detectable label.

11. A method according to claim 1, wherein the target and solid support are of a microarray.

12. A method according to claim 1, wherein the conditions include an amount of a metal ion sufficient to enhance hybridization of the target and probe.

13. A method according to claim 1, wherein the conditions include an amount of a metal ion sufficient to enhance hybridization of the target and probe, wherein the metal ion is selected from the group consisting of Zn++, Ni++, Ca++, Fe++, Fe+++, Mg++, K+, Co++ and Co+++.

14. A method according to claim 1, wherein the solid support comprises a polycationic surface.

15. A method for immobilizing and detecting a polynucleotide probe, comprising the steps of:
contacting a polynucleotide target which is stably associated with a surface of a solid

support, with a double-stranded polynucleotide probe under conditions wherein the double-stranded probe hybridizes with the target; and

detecting specific hybridization of the double-stranded probe to the target.

16. A method according to claim 15, wherein the probe comprises noncovalently linked complementary strands.

17. A method according to claim 15, wherein the probe comprises covalently linked complementary strands.

18. A method according to claim 15, wherein the probe comprises covalently linked complementary strands and is of hairpin structure.

19. A method according to claim 15, wherein the probe comprises covalently linked complementary strands, linked through a first coupling moiety on one strand and a second coupling moiety on the second strand.

20. A method according to claim 15, wherein the probe comprises covalently linked complementary strands, linked through a first coupling moiety on one strand and a second coupling moiety on the second strand, wherein

(a) the first coupling moiety is an alkylamine and the second coupling moiety is selected from an active ester, amide, imine, aldehyde, bromoacetamide and thiocyanate; or

(b) the first coupling moiety is a thiol and the second coupling moiety is selected from a disulfide, maleimides and bromoacetamides.

21. A method according to claim 15, wherein the probe comprises a detectable label.

22. A method according to claim 15, wherein the target is single-stranded.

23. A method according to claim 15, wherein the target and solid support are of a microarray.

24. A method according to claim 15, wherein the solid support comprises a polycationic surface.